Homeland Defense & Security
Information Analysis Center

Biological Agent Detection for the Warfighter

R. Cory Bernhards, Ph.D.

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Research Microbiologist,
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Edgewood Chemical Biological Center

June 21, 2018

The views presented are those of the speaker and do not necessarily represent the views of DoD or its components.

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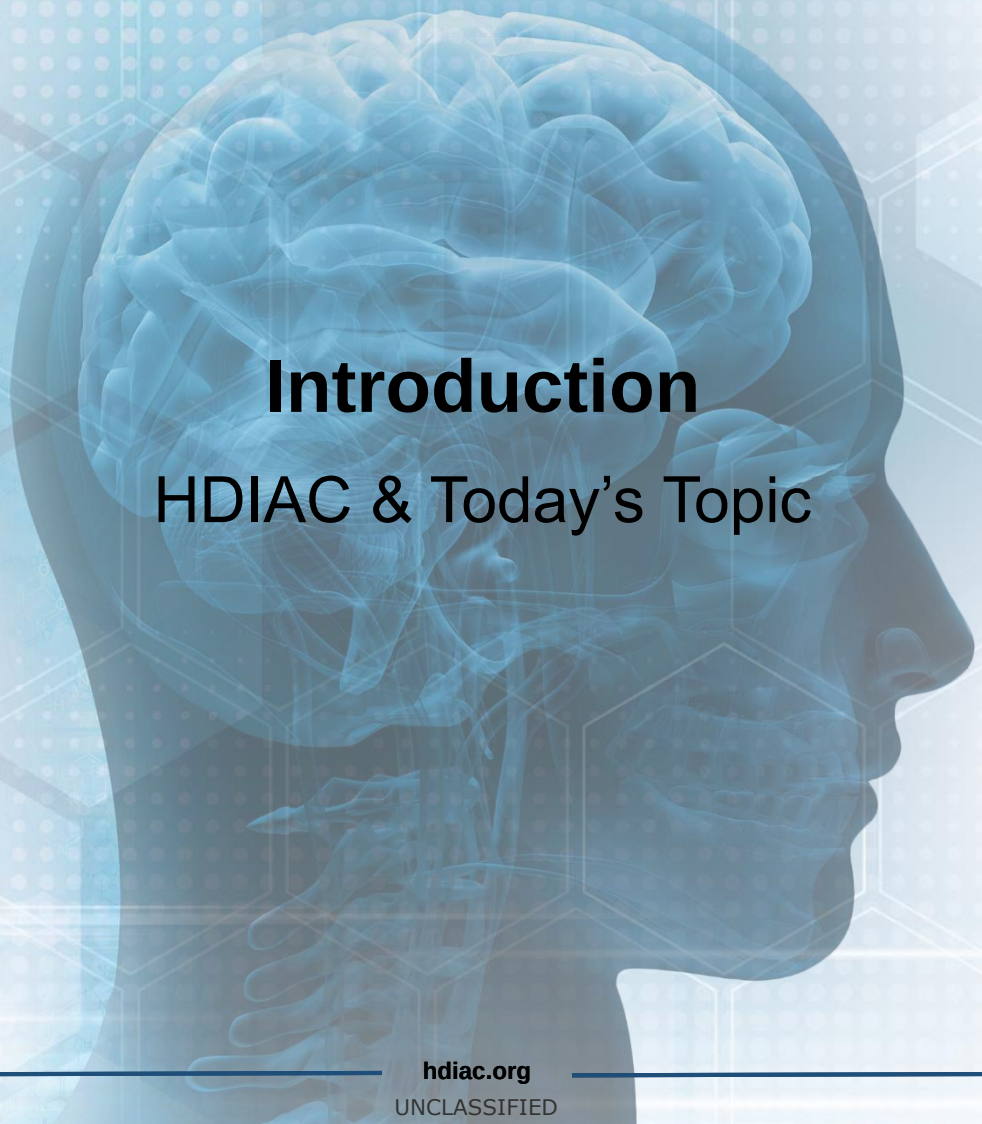

Introduction

HDIAC & Today's Topic

Homeland Defense & Security Information Analysis Center

HDIAC Overview

What is the Homeland Defense & Security Information Analysis Center (HDIAC)?

One of three Department of Defense Information Analysis Centers

Responsible for acquiring, analyzing, and disseminating relevant scientific and technical information, in each of its eight focus areas, in support of the DoD and U.S. government R&D activities

HDIAC's Mission

Our mission is to be the go-to R&D/S&T and RDT&E leader within the homeland defense and security (HDS) community, by providing timely and relevant information, superior technical solutions, and quality products to the DoD and HDS Communities of Interest/Communities of Practice.

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HDIAC Overview

HDIAC Subject Matter Expert (SME) Network

HDIAC SMEs are experts in their field(s), and, typically, have been published in technical journals and publications.

SMEs are involved in a variety of HDIAC activities

- Authoring HDIAC Journal articles
- Answering HDIAC Technical Inquiries
- Engaging in active discussions in the HDIAC community
- Assisting with HDIAC Core Analysis Tasks
- Presenting webinars

If you are interested in applying to become a SME, please visit HDIAC.org or email info@hdiac.org.

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Overview: Biological Agent Detection for the Warfighter

- HDIAC has identified an increased interest among the DoD and HDS communities in mitigating chemical and biological threats to the warfighter
- Advances in science and technology (e.g., synthetic biology) allows for the creation of novel biological agents by adverse actors
- Technologies for *detecting* and *identifying* biological threat agents have evolved in tandem with the above, but generally lag behind methods for novel agent generation
- Innovative and enhanced technologies offer rapid in-theater detection of biological threats—a critical capability for warfighter and force protection

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R. Cory Bernhards, Ph.D.

Research Microbiologist, Defense Threat Reduction Agency, Edgewood Chemical Biological Center

R. Cory Bernhards, Ph.D., (B.S. and Ph.D., biological sciences, Virginia Tech) is a research microbiologist for the Defense Threat Reduction Agency, conducting research at Edgewood Chemical Biological Center at Aberdeen Proving Ground, MD. His research focuses on developing novel detection systems for biothreat agents with an emphasis on rapid sample preparation and field-deployable devices.

Previously, Bernhards conducted an NRC Postdoctoral Fellowship at the U.S. Army Medical Research Institute of Infectious Diseases in Frederick, MD, where he performed research on *Burkholderia* and *Bacillus anthracis*.

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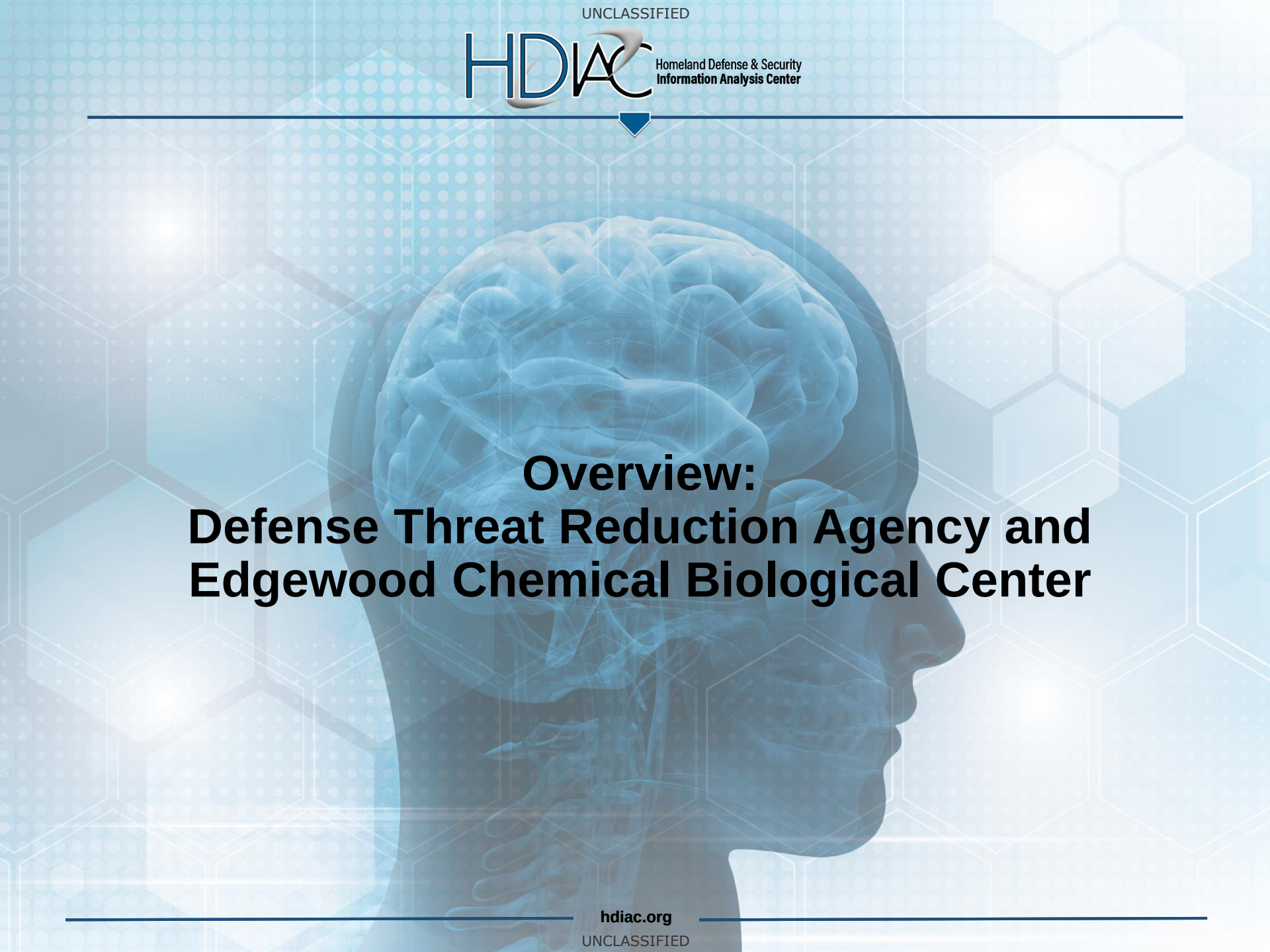

Overview:
Defense Threat Reduction Agency and Edgewood Chemical Biological Center

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Defense Threat Reduction Agency (DTRA)

Mission:
To safeguard the US and its Allies from Weapons of Mass Destruction

- **Chemical**
- **Biological**
- **Radiological**
- **Nuclear**
- **High-Yield Explosives**

by providing capabilities to reduce, eliminate and counter the threat, and mitigate its consequences.

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Biological Threats Expanding in Scope

Biological

- Traditional Biological Threat Agents
- Emerging Diseases
- Enhanced Threats

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DTRA Laboratory Activities

Scientific Positions Include:

Toxicologist **Chemist**

Microbiologist **Pharmacologist**

Computational Biologist

Current Projects:

- **Stem cells and Neurotoxicity**
- **Systems biology/Host-toxicant response**
- **Development of enzyme assays**
- **Development of Viral Evolution program/scripts**
- **Development of a platform that supports DNA/RNA sequence analysis in forward deployed situations**

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Edgewood Chemical Biological Center (ECBC)

Who We Are

We are the nation's primary research and development resource for non-medical chemical and biological defense

What We Do

We couple research and science with engineering and field operations to create new and effective chemical and biological defense solutions

Why We Do It

We do all of this to keep the warfighter, the nation, and the world safe from chemical and biological threats now and in the future

For almost 100 years ECBC has been a unique national asset. We provide innovative and cost-effective chemical and biological defense technology solutions through our scientific and engineering expertise, coupled with our unique facilities and collaboration with partners.

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Why ECBC?

ECBC provides an extraordinary capability against emerging biological and chemical threats by merging intelligence assessments with its world-renowned expertise. ECBC provides a unique blend of distinguished scientists and engineers to research and analyze the emerging threats and technological advances.

Providing forensic, elimination and enhanced detection solutions to soldiers in theatre

Eliminating Syrian chemical stockpiles with the FDHS

Providing product support solutions to help with the detection of the Ebola Virus and transportation of personnel

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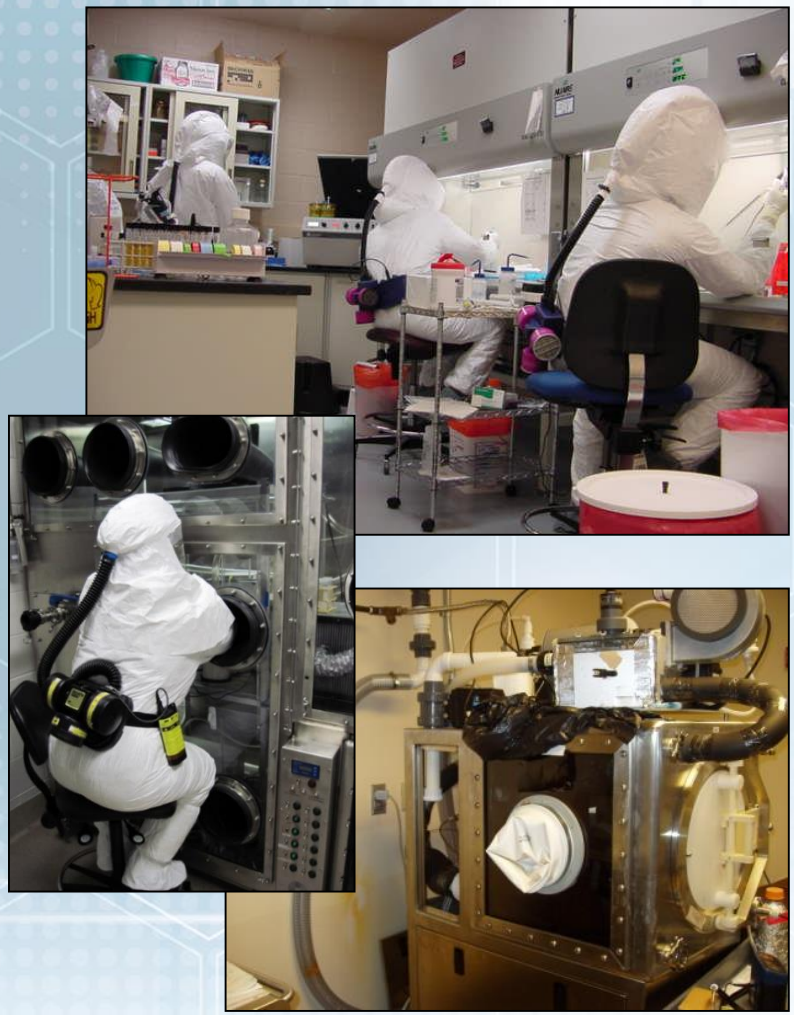
ECBC BioSciences Division

- Biosafety Level 3 (BSL-3) laboratory specializing in decon & detection with bacteria, toxins, and virus
- Aerosolization capabilities available up to BSL-3 for deposition studies, sampling, or challenge testing
- Expertise in quantitative testing of sporicides, fumigants, & disinfectants
- Test and evaluation of COTS/GOTS biodetection suites of hardware
- Biological process engineering
  - Scale-up processing: Ranging from 5 to 1500-liter fermenters
  - Finishing: milling, drying, particle sizing, packaging



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ECBC BioSciences Division

- Sequencing facility with the capability to sequence up to 20 bacterial genomes per week at the draft level
- Next-generation sequencing (NGS) platforms including Illumina HiSeq, MiSeq, and Oxford Nanopore MinION
- Genomic and Proteomic cores with an integrated laboratory information management system (LIMS), experienced technical staff, and computational support
- Contemporary molecular biology, microbiology and biochemistry techniques

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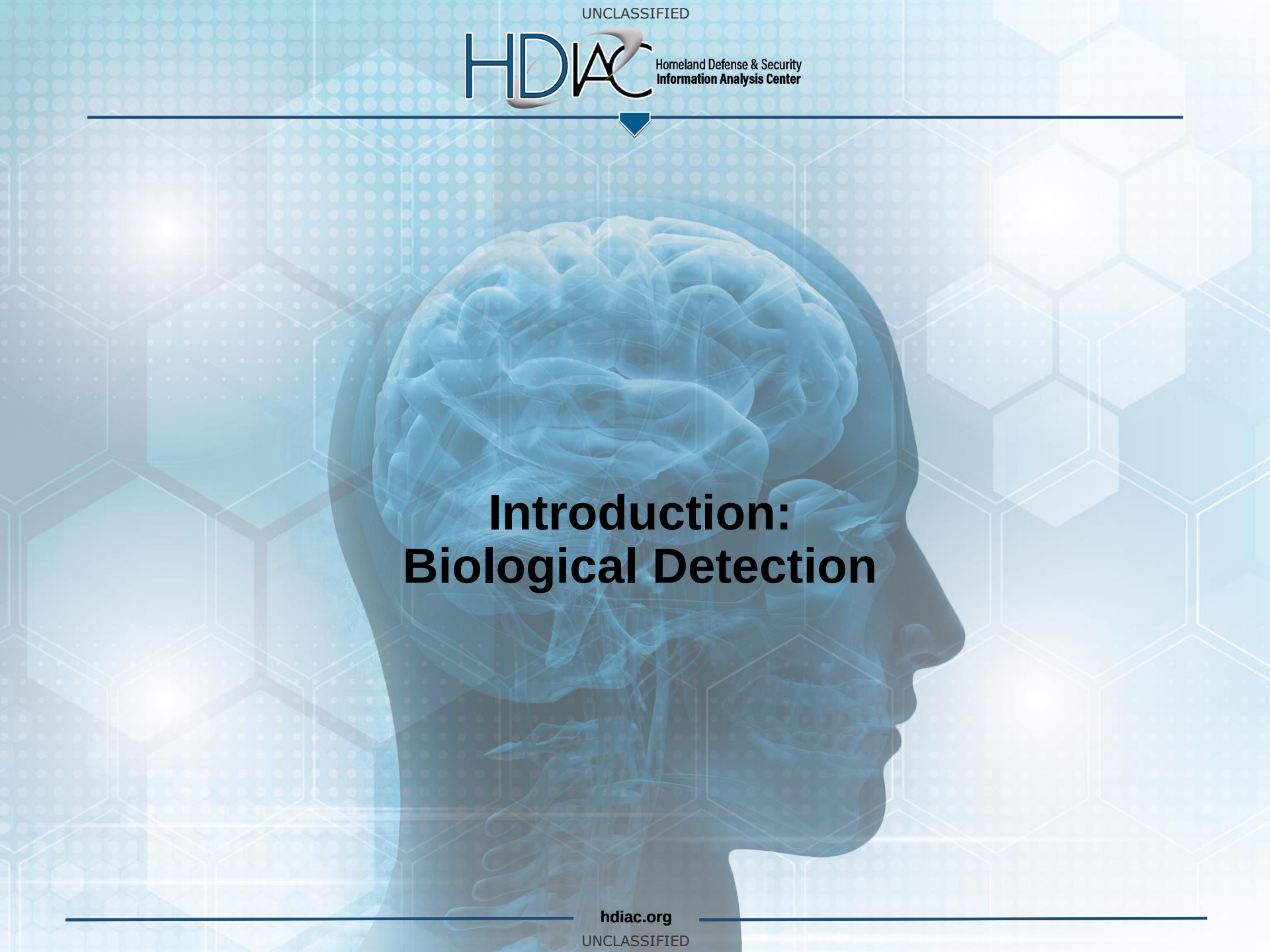


Introduction: Biological Detection

Recent Advancements

- Computational Power
  - Computing power/performance continues to grow at an exponential rate
- Communication Capabilities
  - Near-field wireless and smartphone-based applications have aided and enhanced the design of fieldable chemical and biological detection devices
- Microfluidics
  - There has been a surge in the development of microfluidic devices in the past few decades for biological detection, especially for point-of-care diagnostics

Fieldability

- Reducing Size, Weight, and Power (SWaP)
  - Together, SWaP characteristics ultimately classify the feasibility of a technology's deployment
- Sensitivity
  - Ability to detect an agent at a given concentration(s)
- Specificity
  - Ability to identify/distinguish detected agents from each other
- Sample Throughput
  - Rate of sample analysis for detection and/or identification

Matrix Complexity

There are a large number of matrices for both environmental and clinical sample types that need to be analyzed for biological detection purposes, and each one can present unique challenges.

Environmental

- Water sampling (drinking water, wastewater)
- Plants/vegetation
- Vectors (e.g., mosquitoes)
- Aerosols (air)
- Culture media
- Suspicious powders
- Soil

Clinical

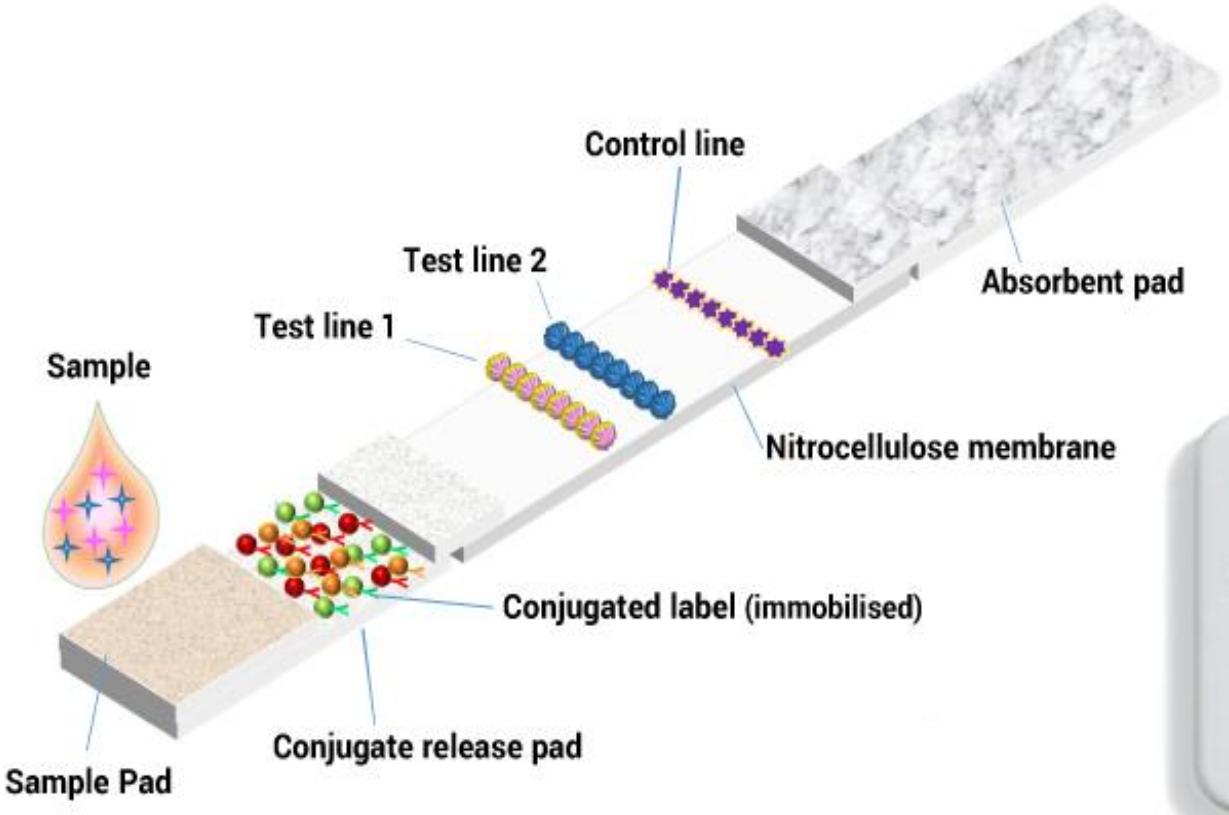
- Blood
- Plasma
- Urine
- Cerebrospinal fluid (CSF)
- Sputum
- Saliva
- Stool

Types of Biological Detection

- Environmental Detection vs. Diagnostics
  - Diagnostic testing on clinical samples requires a higher level of scrutiny when compared to environmental detection
- Two main types of detection technologies
  - Antibody-based detection
    - Lateral flow immunoassays, plate-based assays, microbead-based assays, biosensors, etc.
  - Molecular detection (nucleic acid-based)
    - PCR, isothermal amplification, sequencing, etc.

Antibody-based Detection

Lateral Flow Immunoassay (LFI)



IMASS

Antibody-based Detection

Luminex MagPix

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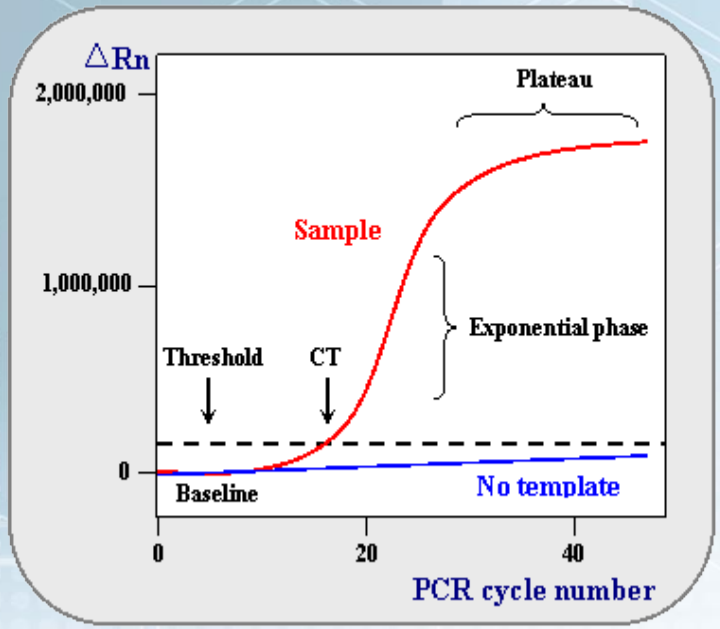
Molecular Detection

Real-time PCR

- Uses 2 primers designed for your target
- Cycles through different temperatures



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Field-deployable Molecular Detection

PCR-based

BioFire RAZOR EX

BioFire FilmArray

Epistem GeneDrive

Biomeme two3

(Three9 version in development)

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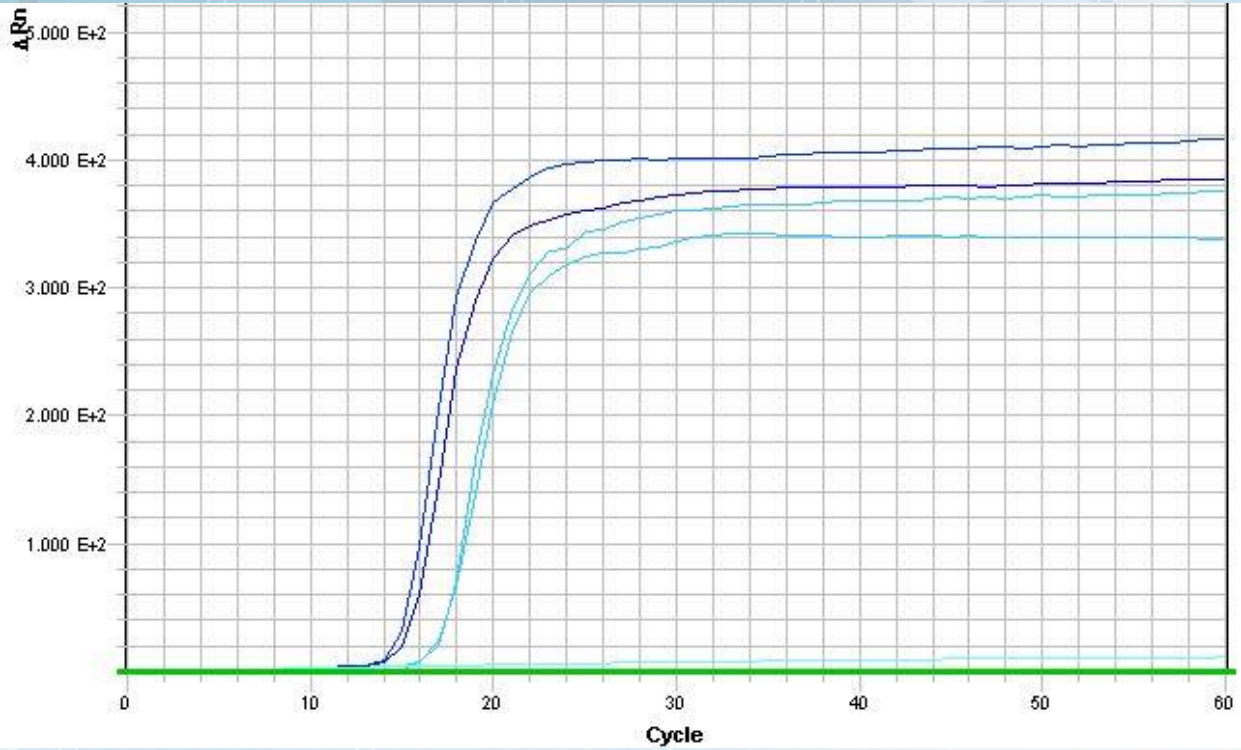
Isothermal Molecular Detection

Loop-mediated Isothermal Amplification (LAMP)

- Uses a set of 6 primers designed for your target
- Works at a constant temperature of around 60°C



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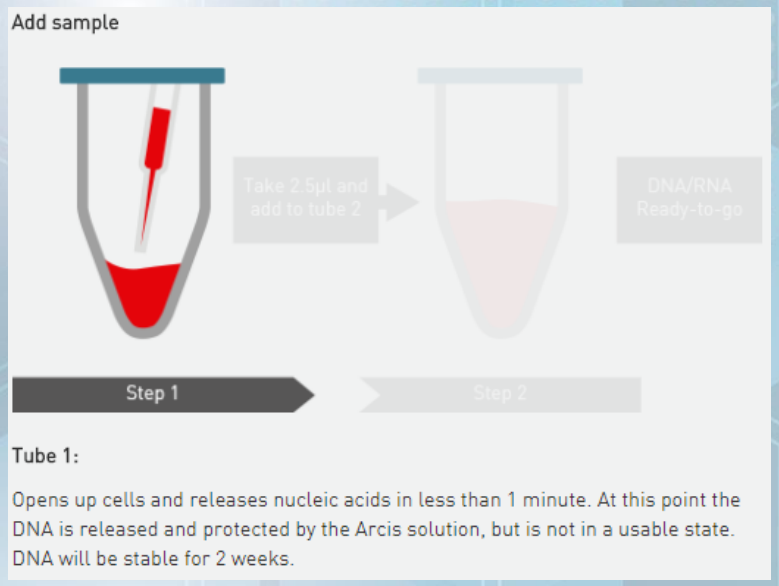
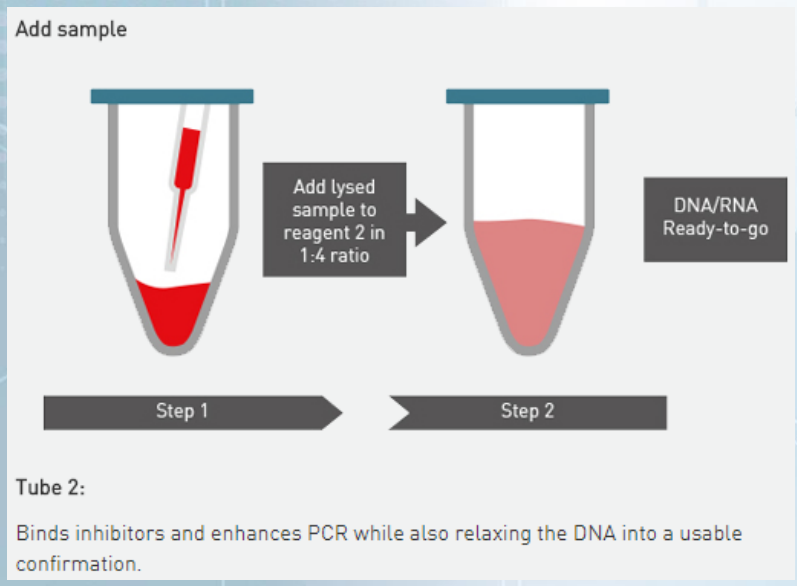
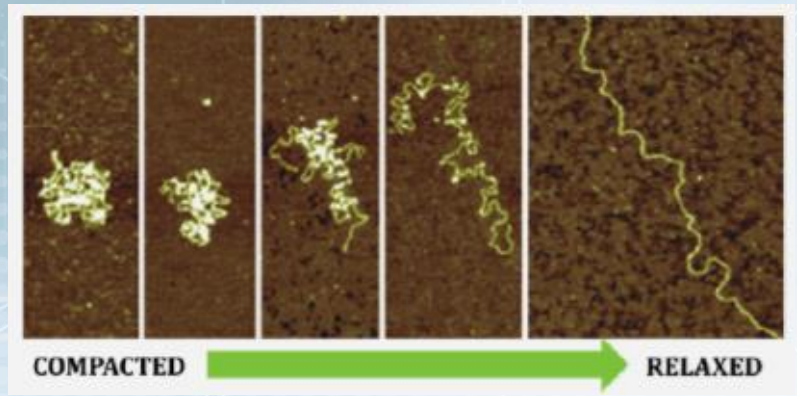
ARCIS Rapid DNA Sample Prep





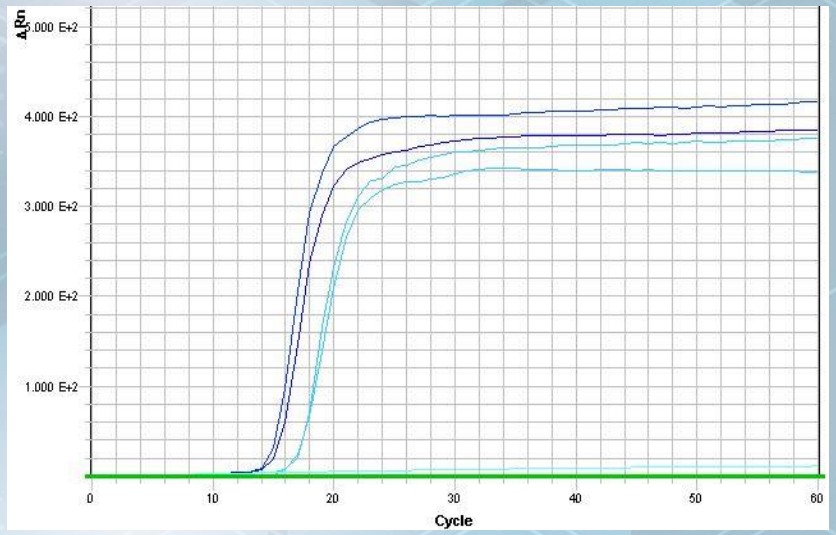
- Simple 2-step process
- Only takes 3 minutes
- No instrumentation required
- Reagents shipped and stored at room temperature
- Works by relaxing the DNA
  - No extraction is needed



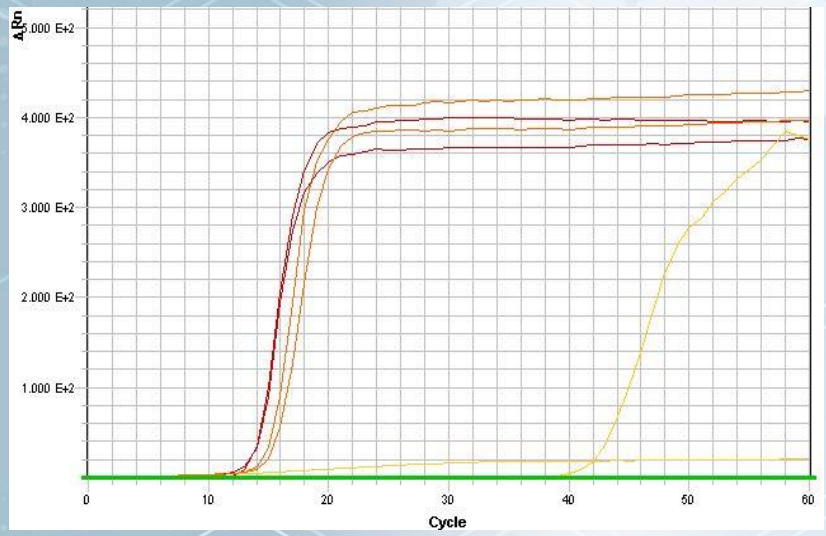
http://www.arcisbio.com/technology-application/

Burkholderia pseudomallei LAMP Detection From Soil

ARCIS

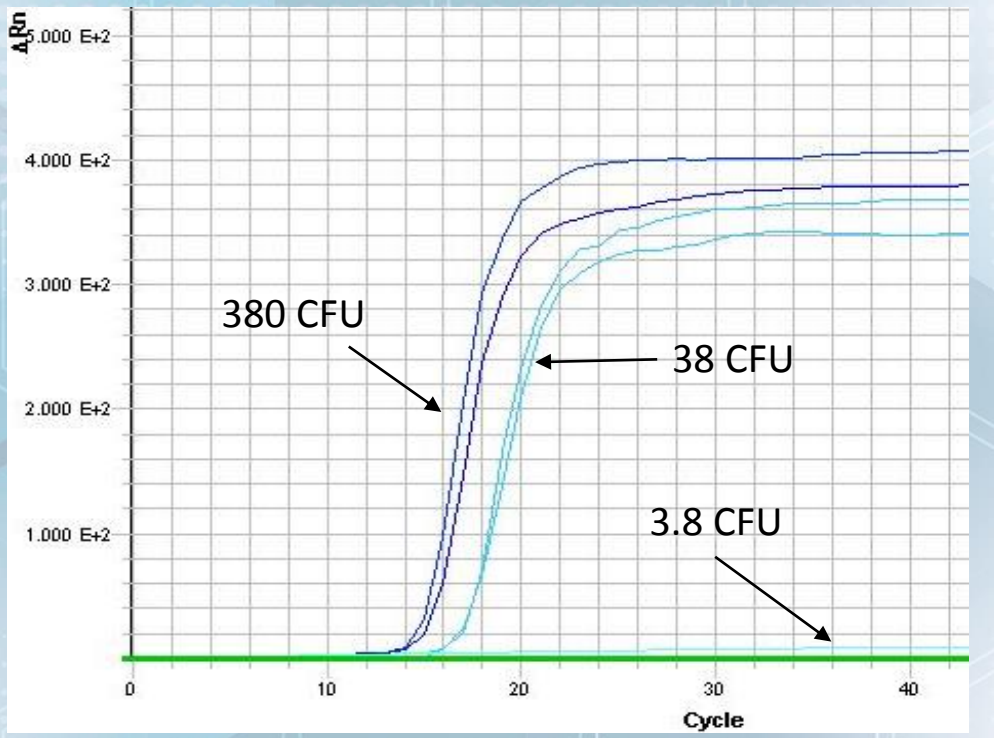


PowerSoil



- Used supernatant from spiked/liquefied soil with *B*. *pseudomallei* strain 82, after 15 min settlement performed ARCIS and PowerSoil preps
- Each sample was analyzed by isothermal real-time LAMP assay specific for *B*. *pseudomallei*

Determining Limit of Detection



B. pseudomallei detection in soil using ARCIS prep and RT-LAMP

- A titration of *B. pseudomallei* was spiked into irradiated Sassafras Sandy Loam (SSL) soil samples, liquefied with 0.01 M $CaCl_2$, and the DNA was prepared using the ARCIS kit.
- RT-LAMP assay was performed. The LOD was estimated to be between 3.8–38 cells.

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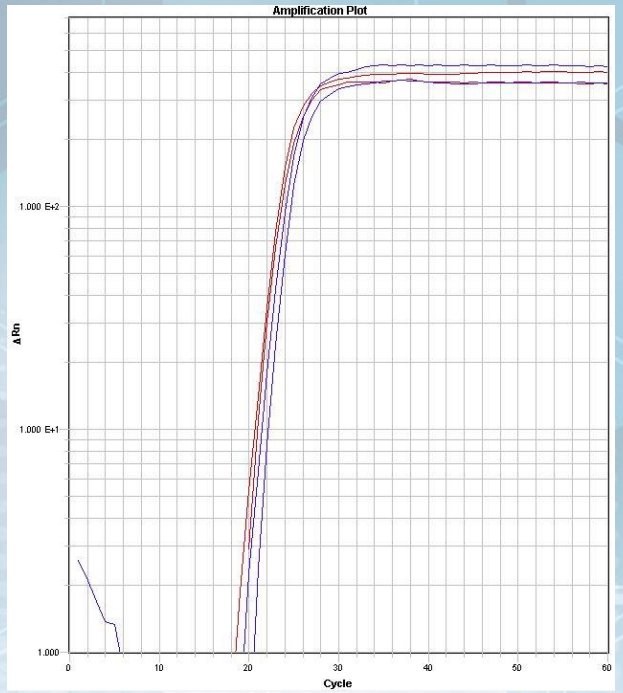
Detection in Humus Soil

Tested the ARCIS kit on spiked samples of humus soil

- Humus soil contains close to 100% organic matter
  - Humic acid comes from the organic matter

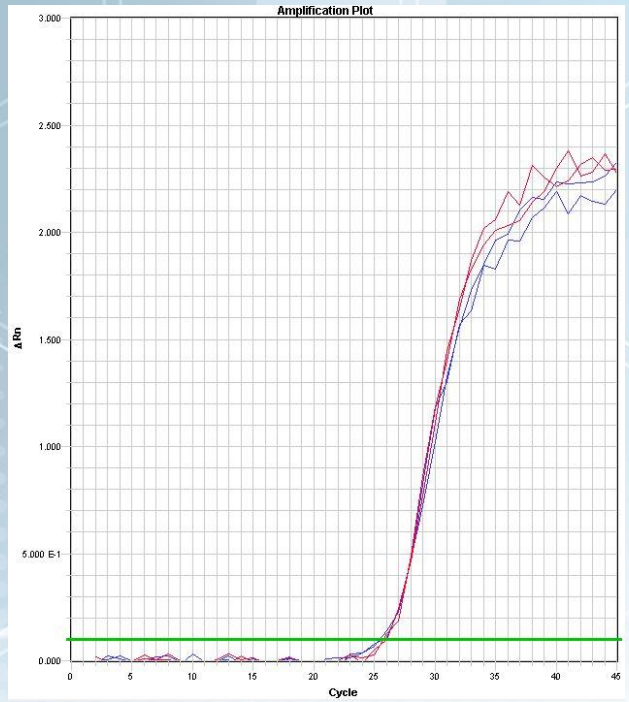
RT-LAMP



Humus soil spiked with *B. pseudomallei*

Positive control (*B. pseudomallei*, no soil)

RT-PCR



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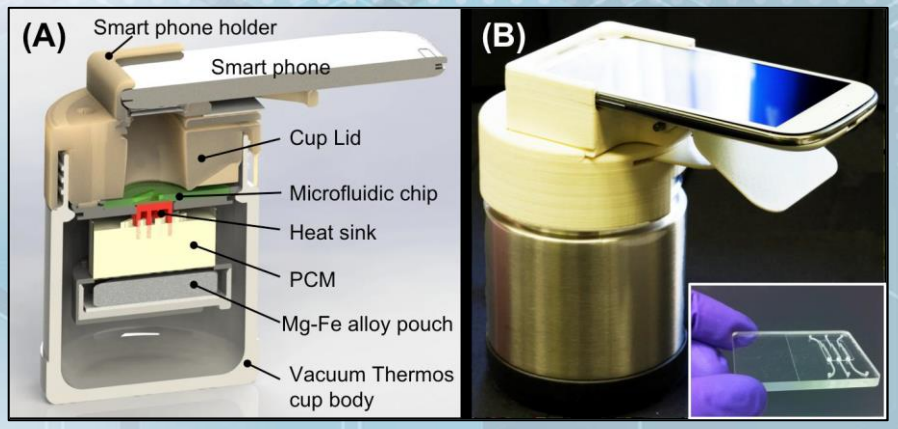
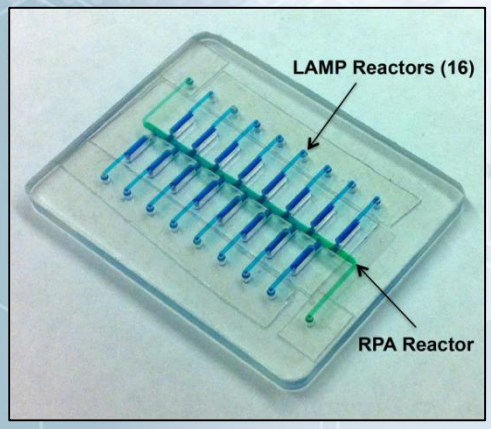
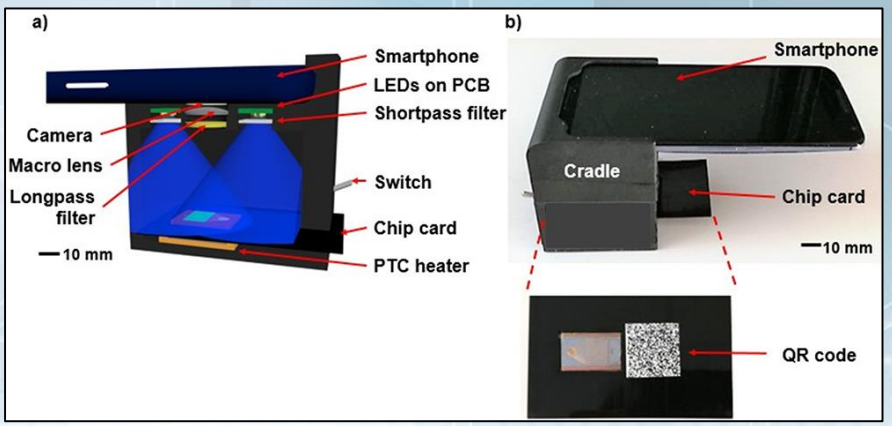
Field-deployable Molecular Detection

LAMP-based



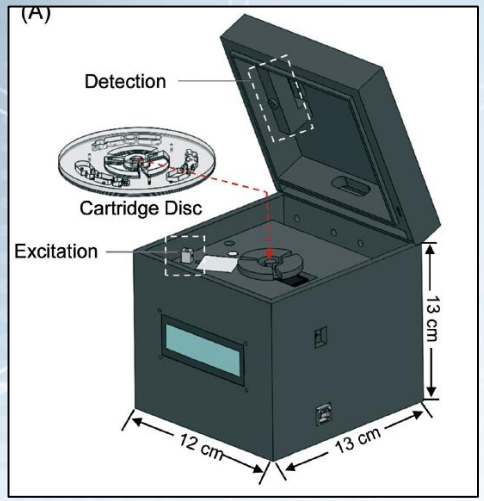


University of Pennsylvania



University of Illinois

AnyMDx
Penn State



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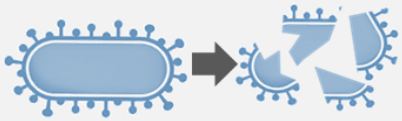
Paper-based Detection

Gather Ingredients

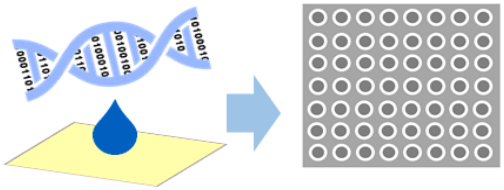


Harvest cellular contents

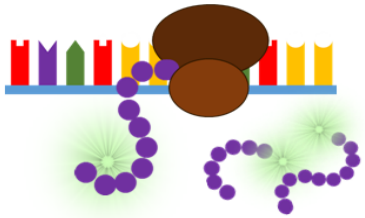
Enrich

Add DNA circuit to *in vitro* expression extract

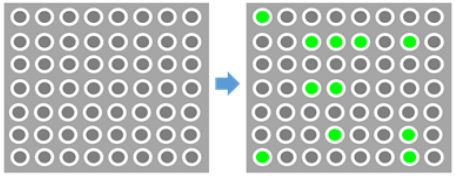
Embed and Preserve



Spot cell-free mixture and DNA circuit onto paper and freeze dry



Short-term cell-like function in the field upon rehydration



Signal in presence of trigger

Fieldable Paper-based Gene Circuit Applications

Medicine

Biological and chemical agent detection

Austere Environments

Agriculture

Harnessing the Power of Cells in a Non-Living Format

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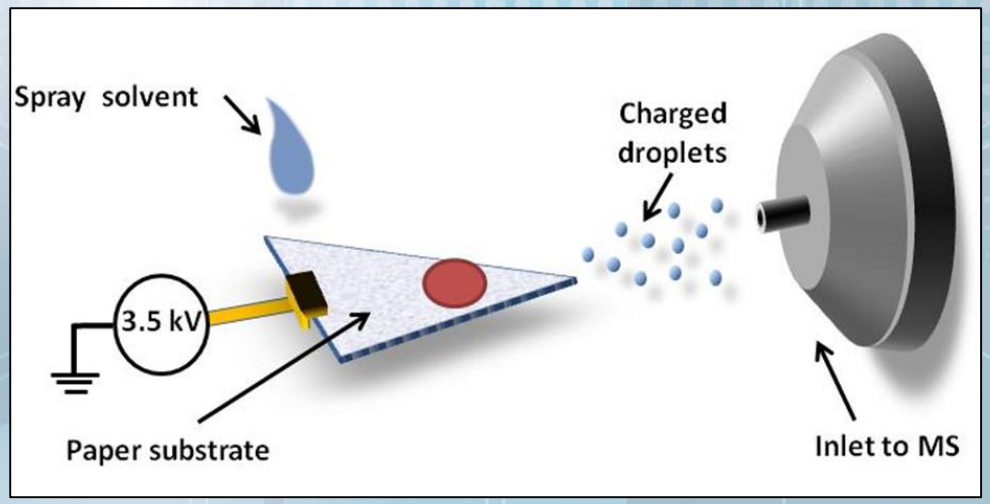
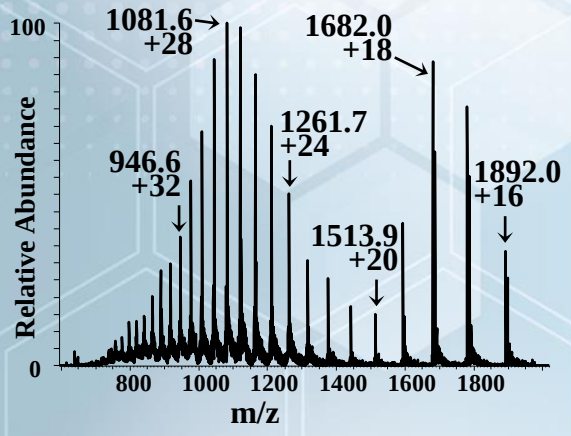
Mass Spectrometry

Detection of Peptides From Proteins Including Biological Toxins

Paper Spray Mass Spectrometry



(a)

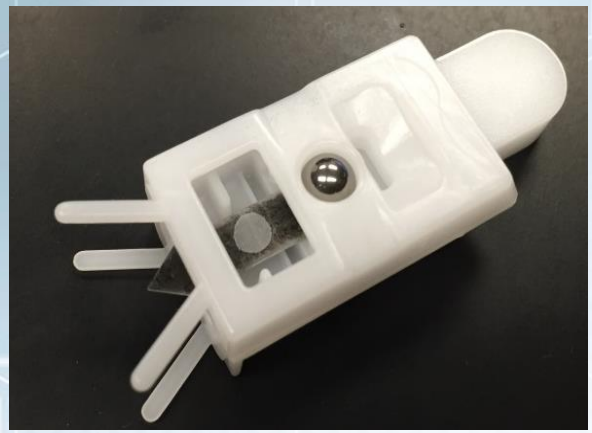


Paper Spray (PS) is an ambient ionization technique, requiring little to no sample preparation, and quantitative in complex backgrounds.

It has been used to detect pharmaceutical and illicit drugs, pesticides, and explosives.

Currently being developed for biodetection.

(b)



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Next-generation Sequencing (NGS)

MiSeq

HiSeq

PacBio

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MinION Portable Nanopore Sequencer

Oxford Nanopore Technologies

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Sample Preparation for MinION Sequencing

VolTRAX

Zumbador

ARCIS

Omnilyse – Claremont BioSolutions

MinION Developments

GridION

PromethION

Flongle

SmidgeION

Future Outlook/Emerging Issues

- Isothermal amplification devices
- UAVs
- Portable sequencing
- Empowering the Development of Genomics Expertise (EDGE)
- Detecting unknown and genetically modified organisms

Conclusion

Conclusions

- While PCR- and isothermal amplification-based technologies will continue to be developed for biothreat detection, the power of fieldable genomic sequencing is far superior
- Rapid and accurate detection and identification of biological threats is critical in meeting national security objectives
- Emerging technologies allow for decision-making that is quicker and more informed
- This promotes both warfighter protection and mission readiness

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Questions?

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HDIAC Services

Technical Inquiry Service

- HDIAC provides up to 4 free hours of information services:
  - Literature searches
  - Document/bibliography requests
  - Analysis within our eight focus areas – Alternative Energy, Biometrics, CBRN Defense, Critical Infrastructure Protection, Cultural Studies, Homeland Defense and Security, Medical, Weapons of Mass Destruction

Core Analysis Task (CAT)

- Challenging technical problems requiring more than 4 hours of research can be solved by initiating a CAT:
  - Pre-competed and pre-awarded
  - Work can begin on a project approximately two months after the statement of work has been approved
  - Cap of $500,000 (through August 31, 2018)
  - Must be completed within 12 months

For more information: https://www.hdiac.org/technical_services

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Thank You